

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US2004/038808

International filing date: 19 November 2004 (19.11.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/523,637
Filing date: 20 November 2003 (20.11.2003)

Date of receipt at the International Bureau: 22 May 2008 (22.05.2008)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

74533

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 22, 2008

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/523,637

FILING DATE: November 20, 2003

RELATED PCT APPLICATION NUMBER: PCT/US04/38808

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS US60/523,637



Certified by

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

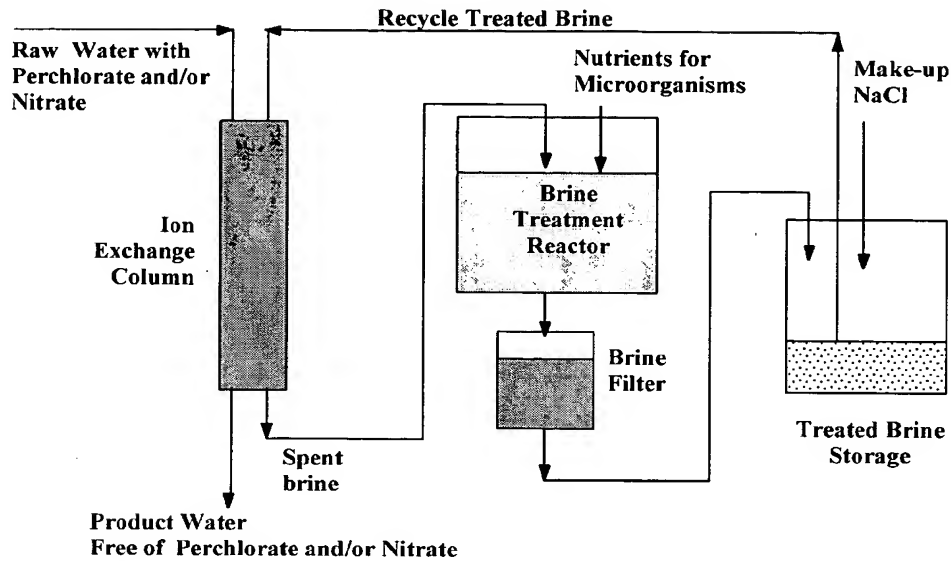
ABSTRACT

5 Our research has led to the development of a novel method to stabilize biological treatment systems in high saline (brine) solutions. Through our research, first directed towards the destruction of perchlorate in ion-exchange brines, we have discovered that through the addition of divalent cations, e. g., Mg^{2+} or Ca^{2+} , to increase the divalent/monovalent cation ratio, we can create conditions that will allow microorganisms to degrade pollutants (in this case perchlorate) as rapidly and as stably as if they were in freshwater cultures.

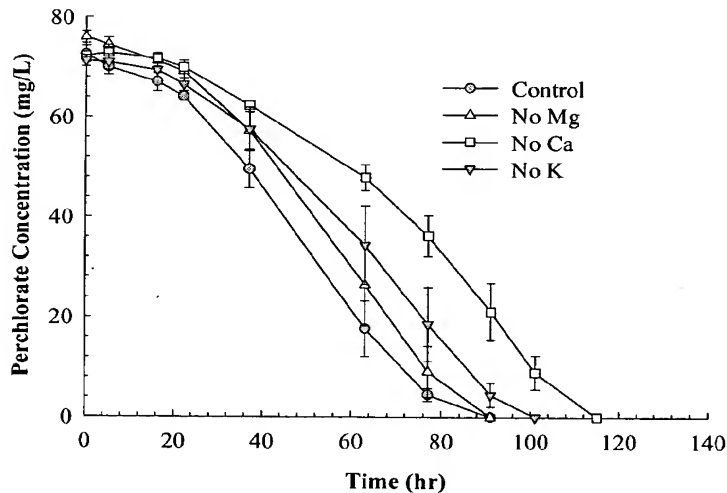
Claims

We Claim:

- 5 1. The addition of divalent cations such as, but not limited to, Ca^{2+} or Mg^{2+} to wastewaters containing biologically degradable compounds and high concentrations of monovalent cations such as, but not limited to, Na^+ or K^+ to stabilize the growth and degradation properties of the cultures.
- 10 2. A biological method for the treatment of brine contaminated with perchlorate, nitrates, and other anions removed from contaminated water comprising:
 - (a) providing an anoxic/anaerobic biological reactor containing a mixed bacterial culture capable of reducing perchlorate and nitrate;
 - (b) maintaining an anoxic/anaerobic environment in the reactor by one or more of the following methods; (i) keeping the reactor and brine system sealed; (ii) sparging or
 - 15 purging the reactor with oxygen-free gas such as, but not limited to, nitrogen or argon;
 - (c) maintaining suitable nutrient and environmental conditions in the anoxic/anaerobic reactor such as the addition of a defined energy source such as H_2 (as H_2 gas or other hydrogen delivery chemicals) or an organic energy source such as, but not limited to, acetate, ethanol, methanol, or lactate in amounts \geq the stoichiometric requirements of
 - 20 electrons required to reduce the perchlorate and nitrate present and for microbial growth, and
 - (d) the addition of a divalent cation such as but not limited to Mg^{2+} to keep the divalent cation to Na^+ ratio ≥ 0.05 mole/mole.
- 25 3. The method of claim two where the biological culture is fed the wastewater in a sequencing batch reactor mode or as a continuous culture operated as a plug flow, dispersed plug flow, or continuously stirred tank reactor, or as a packed, expanded, or fluidized bed column.
- 30 4. The method of claim two where a solid medium, including but not limited to; diatomaceous earth, activated carbon, sand, or ion-exchange resin, is added as a solid support for microbial growth.
- 35 5. All inventions substantially as described herein

FIGURES

5 **Figure 1. The perchlorate nitrate ion-exchange process with brine treatment and reuse**



10 **Figure 2 Perchlorate Removal in Media Deficient of Individual Cations: 6% Culture 2nd Transfer.**
 A 10% transfer of the 6% NaCl synthetic medium culture was used to inoculate media deficient in individual cations. After the initial allotment perchlorate was degraded, the cultures were then used to inoculate a second set of cultures in media deficient in individual cations. The data points represent the average of three replicates, and the error bars present one standard deviation.

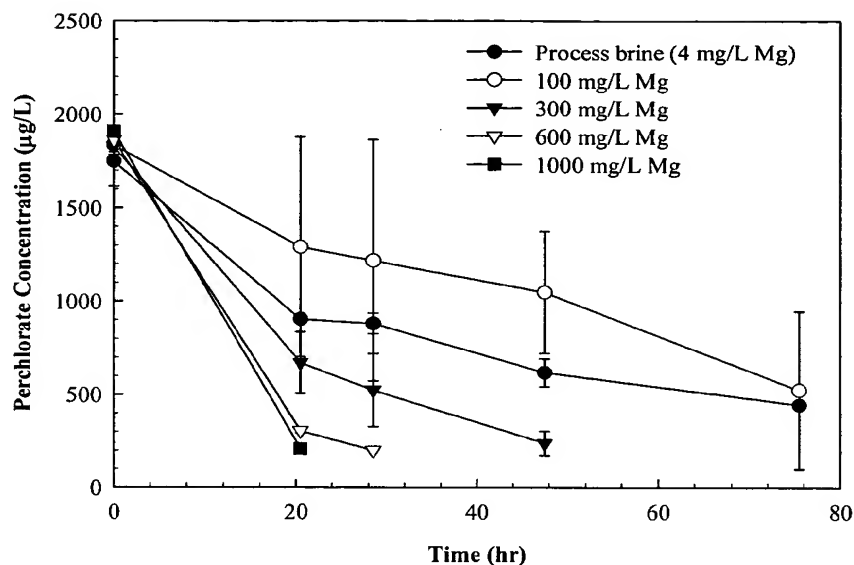


Figure 3 The Effect of Increasing Mg^{2+} Concentration on Perchlorate Degradation in a 3% NaCl Brine

Perchlorate nitrate ion-exchange process brine was amended with increasing amounts of perchlorate and inoculated with a concentrated and washed sample of a 3% NaCl culture. The data points represent the average of three replicates, and the error bars correspond to one standard deviation. The molar ratios of Mg/Na are as follows: 100 mg/L Mg = 0.008; 300 mg/L Mg = 0.024; 600 mg/L Mg = 0.048, 1000 mg/L Mg = 0.08.

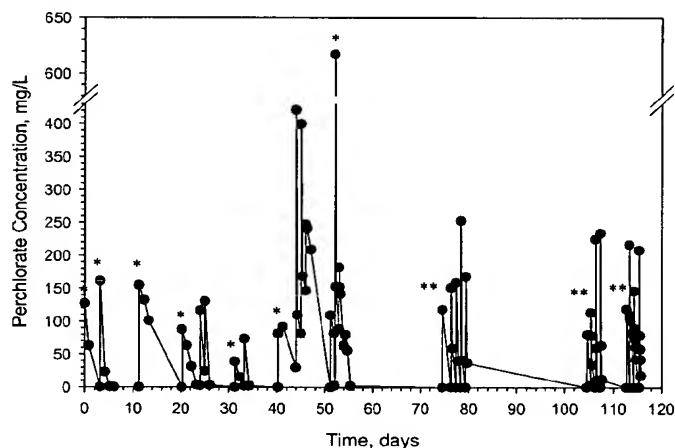


Figure 4. Performance of the culture fed perchlorate and nitrate at 6% NaCl.

The * indicate SBR operation while increases in perchlorate concentration not marked with an asterisk are due to spikes of concentrated perchlorate solution. The feeds marked with the * contain a ratio of Mg^{2+}/Na^{+} of 0.05 mol/mol, while feeds marked with ** indicate a Mg^{2+}/Na^{+} of 0.1 mol/mol.

DOCKET NO. 123029-1032 (UHID #2004-007)

PROVISIONAL PATENT

EXPRESS MAIL NO. EV 322136136 US

DATE OF DEPOSIT: November 20, 2003

This paper and fee are being deposited with the U.S. Postal Service Express Mail Post Office to Addressee service under 37 CFR § 1.10 on the date indicated above and is addressed to Mail Stop Provisional Patent Application, Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450.

Marisa Sweeney

Name of person mailing paper and fee


Signature of person mailing paper and fee

UNITED STATES PROVISIONAL PATENT APPLICATION

for

**Method for Stabilization of Biological Cultures to Allow Biological
Treatment of Brines**

Inventor(s): Dr. Deborah J. Roberts; Houston, Texas
Dr. Dennis Clifford; Houston, Texas
Dr. Xiaohua Lin; Houston, Texas
Dr. Thomas Gillogly; Pasadena, CA
Mr. Lee Aldridge; South Pasadena, CA
Mr. Stewart Lehman, Sierra Madre, CA

Assignee: The University of Houston

Attorneys:
Tim Headley
Reg. No. 31,765
Gardere Wynne Sewell LLP
1000 Louisiana, Suite 3400
Houston, Texas 77002-5007
Tel.: 713 276 5320
Fax: 713 276 6320
theadley@gardere.com

UNITED STATES PROVISIONAL PATENT APPLICATION

TITLE: [Method for Stabilization of Biological Cultures to Allow
Biological Treatment of Brines]

INVENTORS: Dr. Deborah J. Roberts; Houston, Texas
Dr. Dennis Clifford; Houston, Texas
Dr. Xiaohua Lin; Houston, Texas
Dr. Thomas Gillogly; Pasadena, CA
Mr. Lee Aldridge; South Pasadena, CA
Mr. Stewart Lehman, Sierra Madre, CA

GOVERNMENT SPONSORSHIP

This invention was made in part with government support under Account #2805 awarded by the MWH/AWWARF. This invention was also made in part from the University of Houston under the grant number 1551320 (cost Center # 00730-5022-H0068-B0001-G086414).

BACKGROUND OF THE INVENTION**1. Field of the Invention:**

The present invention relates to the use of a novel process for stabilization of biological cultures to allow the treatment of brine solutions. This invention particularly relates to the addition of divalent cations to the brine such that the resulting divalent/monovalent cation ratio is at 0.05 mole/mole or greater.

2. Description of the Background Art

Many industrial wastes such as ion exchange brines, oilfield production brines, spent caustic, and brines produced during chemical manufacture contain elevated levels of salt concentrations (especially Na^+). These wastes may also contain contaminants that would be amenable to biological treatment if organisms could be found to function in high salt wastes. Alva and Peyton (2003) state "We foresee an increasing need for the use of biological treatment adapted to saline and alkaline environments in industrial wastewater systems" they also point out that "it is known that traditional pollutant biodegradation is less efficient or does not function when salinity increases above seawater levels".

The literature search has not revealed that any other researchers are familiar with this method for increasing the salt tolerance of cultures. Alva and Peyton (2003) examined

the growth and phenol degradation in their cultures at different salt concentrations and did not increase the concentrations of divalent cations (Ca^{2+} or Mg^{2+}) when they increased the Na^+ concentration.

5 Logan et al. (2001b) screened six sources of inoculum collected from different salt-water environments for perchlorate reduction. After three months incubation, growth was observed in medium containing perchlorate and 3% NaCl with inocula from only three sources (seawater, saline lake and biofilm/sludge). Two of these three (seawater and saline lake water) grew through 3-7% salinity in subsequent transfers. They make no
10 mention of increasing the divalent cation concentrations when they increased the Na^+ concentrations in their tests.

Coppolla et al. (2000) patented a process for the treatment of perchlorate contaminated briny wastewater but dilute the brine to 3% NaCl and make no mention of the divalent
15 cation concentration in their treatment scheme.

Okeke et al. (2002) obtained cultures that could reduce both perchlorate and nitrate in 0-5% NaCl environments. Again, there was no indication in the manuscript that they changed the concentrations of divalent cations when they increased the sodium
20 concentrations in their salt tolerance tests.

Several other researchers have conducted salt tolerance tests for the growth of many organisms but we have found none that changed the divalent cation concentration when they changed the sodium concentration.
25

Our research has lead to the development of a novel method to stabilize biological treatment systems in high saline (brine) solutions. Through our research, first directed towards the treatment of perchlorate in ion-exchange brines, we have discovered that through the addition of divalent cations (Mg^{2+} or Ca^{2+}) we can create conditions that will
30 allow microorganisms to degrade pollutants (in this case perchlorate) as rapidly and as stably as if they were in freshwater cultures.

DESCRIPTION OF FIGURES AND TABLES

35 **Figure 1** presents a simple schematic of the combined ion exchange and biological treatment process. The entire process is sealed as much as possible to prevent air and the biological reactor is sparged with nitrogen gas to maintain anoxic/anaerobic conditions. The Mg^{2+} is maintained at the optimal ratio to Na^+ for the best culture stability in specific brine solutions by addition to the spent brine storage tank. Makeup sodium chloride is
40 added in the sweet brine storage tank. Alterations to the proposed diagram include the use of continuous culture to replace the batch culture. A media filter immediately follows the biological treatment unit to prevent any organisms that did not settle in the reactor from coming in contact with the resin bed.

45 **Figure 2** Presents data that verifies that the biological culture does not degrade perchlorate as well when either Ca^{2+} , Mg^{2+} , or K^+ are excluded from the synthetic medium containing 60 g/L NaCl.

5 **Figure 3** Presents data showing that when Mg^{2+} is added to an ion exchange brine at different concentrations, the ability to degrade perchlorate rapidly increases with increase in the Mg^{2+} concentration. This data suggests that the minimum concentration of Mg^{2+} required would be 600 mg/L when 30 g/L NaCl is present in the brine (a $\text{Mg}^{2+}/\text{Na}^+$ of 0.05 mole/mole.).

10 **Figure 4.** Presents data that show that when a culture of perchlorate and nitrate reducing organisms were grown in synthetic medium containing 60 g/L NaCl and 1100 g/L Mg^{2+} a ratio of 0.05 mole Mg^{2+} /mole Na^+ perchlorate was degraded to non-detectable levels in 2-8 days, whereas the degradation time was less than one day when the $\text{Mg}^{2+}/\text{Na}^+$ ratio was increased to 0.1 mole/mole.

15 SUMMARY OF THE INVENTION

Our research has lead to the development of a novel method to stabilize biological treatment systems in high saline (brine) solutions. Through our research, first directed towards the treatment of perchlorate in ion exchange brines, we have discovered that through the addition of divalent cations (e.g., Mg^{2+} or Ca^{2+}) to increase the
20 divalent/monovalent cation ratio, we can create conditions that will allow microorganisms to degrade pollutants (in this case perchlorate) as rapidly and as stably as if they were in freshwater cultures.

25 DETAILED DESCRIPTION OF INVENTION

We have developed a novel method to stabilize biological treatment systems in high saline (brine) solutions. Through our research, first directed towards the treatment of perchlorate in ion-exchange brines, we have discovered that through the addition of
30 divalent cations (e.g., Mg^{2+} or Ca^{2+}) to increase the divalent/monovalent cation ratio, we can create conditions that will allow microorganisms to degrade pollutants (in this case perchlorate) as rapidly and as stably as if they were in freshwater cultures.

35 The example below illustrates how we have applied the concept of adjusting the divalent/monovalent cation ratio in a waste brine to make it a more suitable environment for microorganisms to degrade perchlorate and nitrate to prepare the brine for reuse or safe disposal.

EXAMPLE

Example Biological Treatment of Perchlorate- and Nitrate-Contaminated Ion-Exchange Brines

5 Perchlorate (ClO_4^-) is a contaminant found in groundwater that can be removed by ion-exchange processes (Batista et al. 2000, Gu et al. 2000, Tripp and Clifford 2000) that produces a brine contaminated with perchlorate. This brine is largely defined by the amount of NaCl used to regenerate the resin and ranges from as low as 30 g/L NaCl
10 (3%, or 0.5 M) to as high as 90 g/L (9%, 1.5 M). The brine is a waste stream that must be disposed of. The higher the NaCl concentration in the regenerant brine, the smaller will be the volume of brine that is generated. The EPA is about to enact legislation that will forbid the disposal perchlorate-contaminated brine into the environment. The ability to remove nitrate and perchlorate from the brine will allow brine disposal, and more
15 importantly, the recycle of the brine through the ion-exchange process. This will allow conservation of salt and will decrease the disposal costs.

Typical spent brines from ion-exchange processes treating water with 50-100 $\mu\text{g/L}$ perchlorate and 3-20 mg/L nitrate-N would contain 2.5-10 mg/L perchlorate and 150-500
20 mg/L nitrate-N (Tripp and Clifford, (2000) and Najm et. al. (1999).

Clifford and Liu (1993) developed a sequencing-batch-reactor (SBR) denitrification process to treat and reuse nitrate brine containing 3% NaCl. A pilot study using this ion-exchange process with batch biological denitrification and reuse of the spent brine was
25 conducted successfully in McFarland, California in 1994 where spent brine was denitrified and reused 38 times. (Liu and Clifford, 1996). Compared with a conventional ion-exchange process, brine denitrification and reuse reduced the salt consumption by 50 percent and waste discharge by more than 90 percent.

30 Microbial perchlorate reduction under anaerobic conditions has been studied by many researchers (Attaway and Smith, 1993; Herman and Frankenberger, 1999; Logan et al., 2001a; Rikken et al., 1996). Many microorganisms can reduce perchlorate to harmless chloride. Unfortunately, most known perchlorate-reducing microorganisms cannot endure high salinity in the growth media, and usually require less than 2-3% NaCl
35 (Coates et al. (2000) Malmqvist et al. (1994), Michaelidou et al. (2000).

Logan et al. (2001b) screened six sources of inoculum collected from different salt-water environments for perchlorate reduction. After three months incubation, growth was observed in medium containing perchlorate and 3% NaCl with inocula from only three
40 sources (seawater, saline lake and biofilm/sludge). Two of these three (seawater and saline lake water) grew through 3-7% salinity in subsequent transfers. The maximum growth rate for the saline-lake-water enrichment was $0.060 \pm 0.003 \text{ d}^{-1}$ at a salinity of 5% NaCl. The growth rate decreased to $0.037 \pm 0.002 \text{ d}^{-1}$ at a salinity of 11% NaCl, and no growth was observed at salinity over 13% NaCl. Only one data point showing the change
45 of perchlorate concentration in the medium was presented in the paper, that is, perchlorate was found reduced from 592 to 45 mg/L in 3% salinity after 8 weeks while 71 mg/L cell dry weight was produced.

Coppola (1999) Coppola et al. (2000) reported that HAP-1 or a strain of *Wolinella succinogenes* could reduce perchlorate at 2-3% NaCl in the presence of high concentrations of nitrate, sulfate, ammonia and chlorate. The culture could not grow at higher salt concentrations and required the strict maintenance of microaerophilic conditions and the addition of rich nutrients.

Okeke et al. (2002) obtained cultures that could reduce both perchlorate and nitrate in 0-5% NaCl environments. A *Citrobacter* isolate was reported to provide the fastest nitrate and perchlorate removal in conjunction with their Perclace™ culture, removing 46.4% of the perchlorate fed to it in one week. However, because typical ion-exchange columns treating perchlorate and nitrate will be exhausted in less than 24 hours (Tripp and Clifford, 2000; Najm et. al. 2001), a culture should be able to remove nitrate and perchlorate in less than 24 hours to avoid having to store large volumes of brine.

In 1997 an AWWARF advisory group (including Dr. Clifford) met and discussed issuing RFP's for research projects to remove perchlorate from drinking water. One of these projects, suggested by Dr. Clifford, was to use ion exchange to remove the perchlorate from the water followed by a biological process to remove the perchlorate from the ion-exchange regenerant brine, allowing brine recycle. (Figure 1).

After the RFP was issued, Drs. Clifford and Roberts submitted a proposal teamed with Montgomery Watson Harza (MWH), and their team was awarded the project on the strength of their proposal in comparison with the others submitted. The official start date of the research was March 15, 1999. The initial attempts to develop a biological culture that could treat the brine in high salt from a sewage sludge inoculum were not successful. Cultures were obtained that could degrade perchlorate and the nitrate that is also present in the brine, but they could not be adapted to any more than 15 g/L NaCl. When marine sediments were used as an inoculum, we were able to develop two cultures that could degrade perchlorate and nitrate in synthetic media containing 30 or 60 g/L NaCl (Cang, 2001, Cang et al. submitted for publication). These cultures degrade perchlorate and nitrate simultaneously, and require the complete absence of oxygen from the headspace and the media.

A culture developed from marine sediment that was capable of degrading perchlorate and nitrate in 30 g/L NaCl synthetic media in the first feedings of ion exchange brine became unstable after removal of biomass to perform some experiments. This culture was not maintaining or increasing biomass as most biological cultures do. The culture developed from marine sediment and raised in 60 g/L NaCl synthetic medium could not degrade perchlorate at all in 60 g/L NaCl ion-exchange brine.

On September 2, 2001, we designed the first experiment to determine the effects of the ingredients in the synthetic medium that were different from the chemicals that are present in brine. The major differences were that Mg^{2+} , Ca^{2+} , K^{+} were in the medium at levels present in seawater, but we did not know their concentrations in the brine. Several experiments were performed to determine the effects of adding these to brine or leaving

them out of the synthetic medium. Example results are presented in Figure 2. For this experiment, performed in 60 g/L synthetic medium, we determined the change in activity caused by leaving each cation out. The results showed that leaving out Mg^{2+} , Ca^{2+} , or K^{+} did cause a slowing of perchlorate degradation (Figure 2), i.e., was detrimental to the operation of the culture to degrade perchlorate.

The results of the research were presented at a research group meeting in California on July 15, 2002, and in the ensuing discussion of these results, a PAC member mentioned the key to stable operation of the culture might be the maintenance of a divalent to monovalent cation ratio, as opposed to specific concentrations of any of the cations. Immediate attention in the lab and in the pilot plant was then focused on Mg^{2+} and Ca^{2+} additions to increase the divalent/monovalent cation ratio, as well as the determination of which of these would allow a stable culture to grow and at what concentration. We found that the addition of either Ca^{2+} or Mg^{2+} would allow the cultures to reduce perchlorate rapidly and completely in brine. However, because the Ca^{2+} precipitated out of the brine due to the high level of carbonates present, we then turned our attention to the addition of Mg^{2+} , which remained in the brine in spite of the carbonates present. The addition of either cation was beneficial: Ca^{2+} briefly improved the perchlorate destruction rate prior to its elimination by precipitation, whereas Mg^{2+} remained in the brine and improved its long-term performance for perchlorate destruction. We also found that the culture at 60 g/L NaCl requires more Mg^{2+} than the culture at 30 g/L NaCl, especially when nitrate is also present in the culture. These results verify that the requirement is not for a single concentration of Mg^{2+} but for a ratio of Mg^{2+}/Na^{+} . Currently we know that when the ratio is 0.05 mole/mole (Figure 2 and 3) the cultures can reduce perchlorate rapidly at 30 or 60 g/L NaCl, and when the ratio is increased, the culture performance increases as well.

We have developed a novel biological perchlorate destruction process for treating ion exchange brine so that it can be reused or disposed of as non-hazardous waste. Our ion-exchange biological perchlorate destruction process eliminates perchlorate waste brine and conserves regenerant. We have discovered that the key to the biological destruction of perchlorate is to add magnesium to the brine such that the resulting magnesium/sodium or divalent/monovalent cation ratio is ≥ 0.05 mole divalent cation/mole monovalent cation. This is a non-obvious, non-trivial result that took years to develop.

Ca^{2+} can also be added to the cultures and achieve the same effects, but ion exchange brines contain high concentrations of carbonates, and the Ca^{2+} precipitates out. This will require Ca^{2+} to be added each batch, whereas Mg^{2+} does not precipitate out, and is able to persist in the culture and through the recycle process.

REFERENCES:

The following documents are incorporated by reference into this application:

Alva, V.A. and B. M. Peyton. (2003). Phenol and Catechol Biodegradation by the Haloalkaliphile *Halomonas Campisalis*: Influence of pH and Salinity. *Environmental Science and Technology* 37:4397-4402.

- 5 Attaway, H. and Smith, M. (1993) Reduction of Perchlorate by An Anaerobic Enrichment Culture. *Journal of Industrial Microbiology* 12, 408-412.

- Batista, J.R., McGarvey, F.X., and Vieira, A.R. (2000) The Removal of Perchlorate from Waters Using Ion-Exchange Resins. Chapter 13. In: *Perchlorate In The Environment*. Edited by E.T. Urbansky. Vol. 57. Environmental Science Research. Kluwer Academic/Plenum Publishers, New York.
- 10

- Bruce, R.A, Achenbach, L.A., and Coates, J.D. (1999) Reduction of (Per)chlorate by A Novel Organism Isolated from A Paper Mill Waste. *Environmental Microbiology* 1, 319-331.
- 15

Cang, Yu. (2001). M.S. Thesis, University of Houston

- Cang Yu, D.J. Roberts and D. A. Clifford, submitted for publication in Water Research, Nov. 2002, currently under revision for final submission for publication.
- 20

Clifford D. and Liu, X. (1993) Biological Denitrification of Spent Regenerant Brine Using a Sequencing Batch Reactor. *Water Research* 27, 1477-1484.

- 25 Coates, J.D., Michaelidou, U., Bruce, R.A., O'Connor, S.M., Crespi, J.N., and Achenbach, L.A. (2000) The Diverse Microbiology of (Per)chlorate Reduction. Chapter 24. In: *Perchlorate In The Environment*. Edited by E.T. Urbansky. Vol. 57. Environmental Science Research. Kluwer Academic/Plenum Publishers, New York.

- 30 Coppola, E. (1999). Biodegradation of Perchlorate. Presented at Perchlorate Conference. March 18-19, Ontario, California.

- Coppola et. al. (2000). US Patent No. 6,077,432 "Biodegradation of ammonium perchlorate, nitrate, hydrolysates and other energetic materials".
- 35

- Gu, B.H., Brown, G.M., Alexandratos, S.D., Ober, R., Dale, J.A. and Plant, S. (2000) Efficient Treatment of Perchlorate (ClO_4^-)-Contaminated Groundwater with Bifunctional Anion Exchange Resins. Chapter 16. In: *Perchlorate In The Environment*. Edited by E.T. Urbansky. Vol. 57. Environmental Science Research. Kluwer Academic/Plenum Publishers, New York.
- 40

Herman, D.C. and Frankenberger, W.T. Jr. (1999) Bacterial Reduction of Perchlorate and Nitrate in Water. *Journal of Environmental Quality* **28**, 1018-1024.

- 5 Kengen, S.W.M., Rikken, G.B., Hagen, W.R., Van Ginkel, C.G. and Stams, A.J.M. (1999) Purification and Characterization of (Per)chlorate Reductase from the Chlorate-Respiring Strain GR-1. *Journal of Bacteriology* **181**, 6706-6711.

- 10 Liu, X. and Clifford, D.A. 1996. Ion Exchange with Denitrified Brine Reuse. *Journal American Water Works Association*. Nov, 88-100.

Logan, B.E., Zhang, H.S., Mulvaney, P., Milner, M.G., Head, I. M., and Unz, R.F. (2001a) Kinetics of Perchlorate- and Chlorate-Respiring Bacteria. *Applied and Environmental Microbiology* **67**, 2499-2506.

- 15 Logan, B. E., Wu, J. and Unz, R.F. (2001b) Biological Perchlorate Reduction in High-Salinity Solutions. *Water Research*. **35**, 3034-3038.

- 20 Malmqvist, A., Welander, T., Moore, E., Ternstrom, A., Molin, V., and Sternstrom, I. (1994) *Ideonella Dechloratans* Gen. Nov., Sp. Nov., A New Bacterium Capable of Growing Anaerobically with Chlorate as An Electron Acceptor System. *Applied Microbiology* **17**, 58-64.

- 25 Michaelidou, U., Achenbach, L.A., and Coates, J.D. (2000) Isolation and Characterization of Two Novel (Per)chlorate-Reducing Bacteria from Swine Waste Lagoons. Chapter 25. In: *Perchlorate In The Environment*. Edited by E.T. Urbansky. Vol. 57. Environmental Science Research. Kluwer Academic/Plenum Publishers, New York.

- 30 Najm, I., Trussell, R.R., Boulos, L., Gallagher, B., Bowcock, R., and Clifford, D. (1999) Evaluating Ion Exchange Technology for Perchlorate Removal. *Proceedings of the 1999 AWWA Annual Conference, Water Research*, American Water Works Assoc., Denver, CO.

- 35 Okeke, B. Giblin, T. and Frankenberger W.T. Jr. (2002) Reduction of perchlorate and nitrate by salt tolerant bacteria. *Environmental Pollution* **118**, 357-363.

Rikken, G.B., Kroon, A.G.M. and Van Ginkel, C.G. (1996) Transformation of (per)chlorate into chloride by a Newly Isolated Bacterium: Reduction and Dismutation. *Applied Microbiology and Biotechnology* **45**, 420-426.

- 40 Song, Y. and Logan, B.E. (2001) Poster: Respiratory Pathway Used by Perchlorate Respiring Microorganism (Q-256). ASM Meeting, May. 2001. Orlando, Florida.

5 Tripp, A.R. and Clifford, D.A. (2000) The Treatability of Perchlorate in Groundwater Using Ion-Exchange Technology. In: *Perchlorate In The Environment*. Edited by E.T. Urbansky. Vol. 57. Environmental Science Research. Kluwer Academic/Plenum Publishers, New York.

10 Van Ginkel, C.G., Rikken, G.B. and Kroon A.G.M. (1996) Purification and Characterization of Chlorite Dismutase: a Novel Oxygen-Generating Enzyme. *Archives of Microbiology* **166**, 321-326.

15 Venkatesh, K.R., Klara, S.M., Jennings, D.L., and Wagner, N.J. (2000) Removal and Destruction of Perchlorate and Other Anions from Ground Water Using the *ISEP*TM System. Chapter 14. In: *Perchlorate In The Environment*. Edited by E.T. Urbansky. Vol. 57. Environmental Science Research. Kluwer Academic/Plenum Publishers, New York.

20 All references cited herein are incorporated by reference. While this invention has been described fully and completely, it should be understood that, within the scope of the appended claims, the invention could be practiced otherwise than specifically described. Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art can appreciate changes and modifications that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter.

Please type a plus sign (+) inside this box → ☐

PTO/SB/16 (5-03)
Approved for use through 4/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

17510 U.S. PTO
001523637

11/20/03

INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Deborah J. Dennis Xiaohua Thomas	Roberts Clifford Lin Gilligly	Houston, TX Houston, TX Houston, TX Pasadena, CA			
<input checked="" type="checkbox"/> Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max) Method for Stabilization of Biological Cultures to Allow Biological Treatment of Brines					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number				Place Customer Number Bar Code Label here	
OR Type Customer Number here					
<input checked="" type="checkbox"/> Firm or Individual Name		Tim Headley			
Address		Gardere Wynne Sewell LLP			
Address		1000 Louisiana, Suite 3400			
City		Houston	State	TX	ZIP 77002-5007
Country		USA	Telephone	713 276 5320	Fax 713 276 6320
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		12		<input type="checkbox"/> CD(s), Number	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		2		<input checked="" type="checkbox"/> Other (specify)	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		Acknowledgment postcard			
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE AMOUNT (\$)	
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number		07-0153		\$80.00	
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input type="checkbox"/> No.					
<input checked="" type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: EPA awarded MWAH/AWWARF Grant No. 1551320 (Cost Center 00730-5022-H0068-B0001-G086414)					

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME

TELEPHONE

Tim Headley

713 276 5320

Date

11/20/03

REGISTRATION NO.

(if appropriate)

Docket Number:

31,765

123029-1032

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

P19SMALL/REV05

PROVISIONAL APPLICATION COVER SHEET

Additional Page

PTO/SB/16 (8-00)
 Approved for use through 10/31/2002. OMB 0651-0032
 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Docket Number	123029-1032	Type a plus sign (+) inside this box →	+
INVENTOR(S)/APPLICANT(S)			
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)	
Lee Stewart	Aldridge Lehman	South Pasadena, CA Sierra Madre, CA	

Number 2 of 2

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.